Appendix IV-1 Single-Species Plant Toxicity Test Evaluations

Section IV.B presented the results of an analysis of single-species plant toxicity tests to provide a compilation of specific growth rate (SGR) versus atrazine concentration (C_{ATZ}) relationships for use in the atrazine assessment methdology. This appendix describes the procedures used in this analysis (emphasizing how different types of information were used to estimate SGR relationships) and provides several representative examples of the analyses.

Depending on the nature and amount of information available in the reports for the toxicity tests, various approaches were needed to estimate toxicity relationships for SGRs (note that some of these approaches assume that SGRs are approximately constant over the time-course of the test):

(a) The preferred data were reported SGRs, or reported initial and final biomasses from which SGRs could be computed, at multiple treatment concentrations so that a regression analysis of SGR vs. C_{ATZ} could by conducted. These analyses were by least-square, nonlinear regression using Version 1.1 of the software package TRAP (Toxicity Relationship Analysis Program) (U.S.EPA Midcontinent Ecology Division, 2006), using the "threshold sigmoidal" model option and the log-transform option for C_{ATZ}. This model option provides a sigmoidal regression function shape, but with finite EC_0 and EC_{100} in contrast to logistic or similar models; this mathematical form is based on the slope of the function having a maximum value at the $\log_{10}(EC_{50})$ and declining linearly to zero at the $\log_{10}(EC_0)$ and $\log_{10}(EC_{100})$. The defining parameters for this function are the control SGR (SGR_C), the $\log_{10}(EC_{50})$ and the maximum relative slope (i.e., the relative slope $d(SGR/SGR_C)/d(\log_{10}(C_{ATZ}))$ at the $\log_{10}(EC_{50})$).

(b) When an EC_{50} for the SGR was provided in the report without sufficient information to conduct a regression analysis to obtain both an EC_{50} and a slope, this EC_{50} was included in the data compilation without a value for the slope.

(c) When data for final, but not initial, biomasses were provided for the control (B_{FC}) and multiple atrazine treatments (B_{FT}) and when information was available regarding the SGR_C for the test system, SGRs for each treatment (SGR_T) were estimated by using the following equation:

$$SGR_T = SGR_C + \frac{1}{t} \cdot \ln\left(\frac{B_{FT}}{B_{FC}}\right)$$

where t is the exposure time at which the biomasses were measured. The estimated SGRs were then subject to the same regression analysis as in **(a)** to provide an EC_{50} and slope that was included in the data compilation. A variation of this method involved using an estimated range for the SGR_C based on other test systems; this resulted in a range of estimated EC_{50}s, which were included in the compilation if the range was not too broad.

(d) When only an EC_{50} for net growth was reported (without the information needed to conduct a regression analysis) and an estimate for SGR_C was also available, the percentage decrease *p* in the SGR at the EC_{50} for net growth was estimated by determining the factor by which control biomass would increase over the test duration given the SGR_C, and then solving for the SGR_P that would produce half of this increase. The value for p would then be calculated as $100(1-SGR_P/SGR_C)$ and the EC_{50} for the SGR would extrapolated from this EC_P based on measured slopes from other studies. This was only done if *p* was in the 25-75% range, so the extrapolation was not great.

(e) When the measured variable was O_2 evolution or ^{14}C fixation rates over a short enough time that biomass did not change signficantly, these were treated as proportional to the SGR if they were measured at test start or could otherwise by normalized to current biomass. If cumulative values for these variables were reported over a period of significant growth, they were treated as comparable to measures of net growth, and converted to values proportional to SGR using equations analogous to those used for net growth (**(a),(c),(d)** above).

(f) When data at multiple times were reported, analyses were conducted at each time, but the compiled EC_{50}s and slopes were for selected times that compromised between reductions in control growth rates that can occur later in exposures and uncertainties in quantifying biomasses that can occur early in exposures.

(g) Data based just on chlorophyll content were not used because the chlorophyll content per cell can change markedly in response to atrazine, leading to markedly different EC_{50}s for chlorophyll than for actual biomass. For example, van der Heever and Grobbelaar (1996) reported effect concentrations to be about 2.5-fold higher when based on chlorophyll than when based on cell numbers or dry weight.

The following are a summary of the evaluations for several tests with *Selenastrum capricornutum* as examples of the evaluation methods.

(1) Gala and Giesy 1990, *Selenastrum capricornutum*

The authors provided tabulated absolute SGRs over 96 hours at multiple treatment concentrations, based on hemacytometer cell counts, providing sufficient data for regression analysis. Data for earlier times were not reported, but authors noted the use of extra nutrients to maintain exponential growth. Concentrations were measured.

Measured (Target) Concentration (µg/L)	Author Measured SGR (1/d)
Control	1.007
64 (60)	0.773
121 (120)	0.508
261 (250)	0.244

499 (500)	0.013
EC_{50}	125
Slope	1.11

(2) van der Heever and Grobbelaar 1996, *Selenastrum capricornutum*

The authors provided figures for relative (to control) SGRs over 24, 48, 72 hours for cell weight, cell count, and chlorophyll a both spectrophotometrically and fluorometrically. SGRs were estimated from the figures. Concentrations were nominal. Chlorophyll had much higher ECs, supporting not using such data. The compiled avlues were for dry weight rather than cell counts because weight per cell varied, although the differences are modest.

Nominal	Author Relative SGR, Cell Counts			Author Relative SGR, Dry Weight		
Conc (µg/L)	1d	2d	3d	1d	2d	3d
1	1.13	1.30	1.22	1.06	1.10	1.00
5	0.98	1.00	0.95	1.00	1.18	1.02
10	0.98	1.11	1.07	0.84	1.02	0.91
50	0.97	0.97	0.97	0.88	1.00	0.93
100	0.95	1.10	1.08	0.83	1.06	0.91
500	0.35	0.30	0.30	0.18	0.30	0.33
1000	0.37	0.34	0.37	0.10	0.10	0.10
5000	0.20	0.12	0.10	0.00	0.00	0.00
EC_{50}	452	355	411	243	342	346
Slope	0.57	0.73	0.82	1.04	1.38	1.12

(3) Hoberg 1991, *Selenastrum capricornutum*

The author provided a data table of cell counts at 1, 2, 3, 4 days at multiple concentrations; initial cell counts were $1 \cdot 10^4$. Concentrations were measured and were stable for 4 days; concentrations were 2X higher than target due to diluting error. SGRs were calculated for each duration and concentration from the counts. Declining control SGRs suggested using day 1, except low cell counts on day 1 created uncertainties; the average of day 2 and day 3 was therefore compiled.

Conc (µg/L)		Author Cell Counts ($/10^4$)				Calculated SGR (1/d)			
Target	Measured	1d	2d	3d`	4d	1d	2d	3d	4d
0	-	10.0	33.0	71.7	105.0	2.30	1.75	1.42	1.16
32	76	5.0	9.3	49.7	101.7	1.61	1.12	1.30	1.16
63	130	2.3	5.0	31.7	27.7	0.83	0.80	1.15	0.83
120	250	1.7	4.0	1.7	2.0	0.53	0.69	0.18	0.17
240	510	0.7	2.3	2.0	1.0	<0.00	0.42	0.23	0.00

490	970	0	0	0	0	-	-	-	-
EC_{50}						109	130	171	163
Slope						1.15	0.66	1.50	2.50

(4) Caux et al. 1996, *Selenastrum capricornutum*

The authors only provided a 4-day EC_{50} based on cell counts (26 µg/L), with no data on actual cell counts at test termination for atrazine treatments. No information was provided on actual treatment concentrations. However, they did report an initial cell count of $1 \cdot 10^4$ and a final control cell count of $1\text{-}2 \cdot 10^6$. Based on the midrange of the final control cell count, the control growth was 150-fold over 4 days, equivalent to an SGR_C of 1.25/day. The authors also reported a probit slope of 4.95 for the cell count versus $\log_{10}C$ relationship, which allowed calculation of other EC_Ps (EC_{16} and EC_{84}, corresponding to ±1 standard deviation in probit equation). The factor increase in cell counts at the various EC_Ps were estimated as $150 \cdot (1\text{-}p)$ and converted to their equivalent SGRs, and a regression analysis of SGR versus cell count ECs was conducted.

p (percent reduction in cell counts)	ECp (µg/L)	Relative Growth (Factor increase)	Calculated SGR (1/d)
0		150	1.25
16	16.4	126	1.21
50	26	75	1.08
84	41	24	0.795
EC_{50} (µg/L)			51
Slope			1.62

(5) Versteeg 1991, *Selenastrum capricornutum*

The author reported an EC_{50} of 100 µg/L for ^{14}C fixation rates measured over 5 minutes after 30 minutes exposure to atrazine. Because the exposure was short enough not to result in disparate biomasses among treatments, it was assumed that these ^{14}C fixation rates were proportional to the SGR, so that 100 µg/L is also an estimate of the SGR EC_{50}. No information was available to calculate a slope for atrazine, but ^{14}C fixation rates were reported for multiple concentrations for simazine, which had a slope of 1.18. Assuming these triazine chemicals would have similar slopes, this slope was included in the compilation for atrazine.

Concentration (µg/L)	^{14}C Fixation Rate Atrazine (Percent of Control)	^{14}C Fixation Rate Simazine (Percent of Control)
0		100
25		104
50		103

175		59
300		38
EC_{50} (µg/L)	100	216
Slope		1.18

(6) Larsen et al. 1986, *Selenastrum capricornutum*

The authors reported EC_{50}s for ^{14}C fixation rates, measured over 2 hours after 24 hours prior exposure to atrazine, to average 43 µg/L (range of 34-53) across three tests. Because the 24 hour prior exposure would result in substantially different biomasses among treatments, this measure is not proportional to the SGR and since fixation was not cumulative over the entire period (26 hours), it is also not proportional to net growth. Rather, this measure is proportional to $SGR \cdot e^{SGR}$, assuming that the measured fixation over the 2 hours is approximately proportional to the SGR for whatever the biomass was at 24 hours (e^{SGR} being the biomass at 24 hours if SGR is in units of 1/day). Based on other studies for this species, the SGR_C averaged 1.4/day with a range of about 1.0-1.8 (Table IV-1). An SGR EC_{50} was calculated for the average estimated SGR_C and both ends of the range as follows:

(a) Use the estimated SGR_C to compute a control value for the function $SGR \cdot e^{SGR}$,
(b) Halve the control value to provide the function value at the reported EC_{50} (43 µg/L),
(c) Solve this function for the value of SGR_P at the reported EC_{50}, where "p" refers to the percentage reduction in the SGR needed for the function to be half of the control value; thus, the carbon fixation EC_{50} of 43 µg/L is an EC_P for the SGR,
(d) Calculate p as $100(1-SGR_P/SGR_C)$, and
(e) Use the average slope of other tests on this species (1.08 from Table IV-1) to estimate the SGR EC_{50} from this EC_P.

Estimated SGR_C (1/day)	$SGR_C \cdot e^{SGRc}$	$0.5*SGR_C \cdot e^{SGRc} = SGRp \cdot e^{SGRp}$	SGR_P (1/d)	p (%)	SGR EC_{50} (µg/L)
1.0	2.72	1.36	0.685	31	68
1.4	5.68	2.84	1.022	27	75
1.8	10.89	5.45	1.375	24	82

(7) Roberts et al. 1988, *Selenastrum capricornutum*

The authors reported the number for the doublings (cell count basis) over 3 days at multiple treatments concentrations (nominal concentrations). The number of doublings was converted to a factor increase, which was converted to an SGR and subject to regression analysis.

Nominal Concentration (µg/L)	Number of Doublings	Relative Growth (Factor increase)	Calculated SGR (1/d)
0	7.13	140	1.65
50	6.64	100	1.53
100	5.08	33.8	1.17
150	4.10	17.2	0.95
EC_{50} (µg/L)			173
Slope			1.08

(8) Radetski et al. 1995, *Selenastrum capricornutum*

The authors reported a 72-h EC_{50} of 118 µg/L based on cell counts in a semistatic microplate well test. They also reported an initial cell count of $2 \cdot 10^4$ and a final control cell count of $6.6 \cdot 10^6$, corresponding to an SGR_C of 1.93/d. At the reported EC_{50}, the final cell count would thus have been $3.3 \cdot 10^6$, equivalent to an SGR of 1.70, corresponding to a 12% reduction from the control value (e.g., the growth EC_{50} is an SGR EC_{12}). This lies outside the criteria adopted for extrapolating SGR EC_{50}s from EC_Ps, so this data was not used.

(9) Abou-Waly et al. 1991, *Selenastrum capricornutum*

The authors reported SGRs for multiple durations and concentrations, but only for chlorophyll measurements. Dry weights were reported to have been measured, but were not reported. These data were not used in accordance with adopted criteria. Reported growth rates and EC50s had complicated relationships to time and exposure concentration, thereby substantiating concerns about using chlorophyll measurements.